3. METABOLISM AND PHARMACOKINETICS

The pharmacokinetics of 1,3-butadiene have been reviewed previously by the U.S. Environmental Protection Agency (U.S. EPA, 1985) and the International Agency for Research on Cancer (IARC, 1986). Data from both in vitro and in vivo studies on the toxic effects of 1,3-butadiene have established that 1,3-butadiene metabolites, not the parent compound, cause these toxic effects. Differences have been noted in the toxic responses to 1,3-butadiene among laboratory species, and understanding the pharmacokinetics of 1,3-butadiene and its metabolites is important in assessing the carcinogenic risk and evaluating other health effects associated with exposure to this chemical. This chapter summarizes the recent research that has provided information on the pharmacokinetics of 1,3-butadiene in several animal species and elucidates the metabolism of 1,3-butadiene, via both in vitro and in vivo studies.

The chemical terminology and units used in the publications reviewed in this chapter have been standardized for consistency. Epoxybutene (EB) is used for 1,3-butadiene monoepoxide, 1,3-butadiene monoxide, 1,2-epoxybutene-3, 1,2-epoxy-3-butene, vinyl oxirane, and 3,4-epoxy-1-butene; diepoxybutane (DEB) is used for 1,2:3,4-diepoxybutane; and butene diol (BD) is used for 1,2-dihydroxybut-3-ene and 3-butene-1,2-diol.

3.1. OVERVIEW OF PHARMACOKINETIC STUDIES

In recent years, considerable data have been generated regarding the pharmacokinetics of 1,3-butadiene in various laboratory species. Although in vitro studies can elucidate possible metabolic products and allow measurements of metabolic reaction kinetic constants under controlled conditions, in vivo studies usually encompass several issues of pharmacokinetics and provide an account of the total disposition of the exposed dose. For 1,3-butadiene, because of the toxicity of the 1,3-butadiene metabolites, in vivo pharmacokinetic studies validated the existence of these metabolites and their metabolic rates of activation and detoxification. Absorption of the parent compound was often assessed either from its distribution in the tissue organs or blood or from its excretion in urine, feces, and exhaled air. Absorption and excretion have also been measured from the presence of 1,3-butadiene metabolites in blood, urine, feces, and exhaled air. Species differences have been observed in the toxic effects of 1,3-butadiene in mice, rats, and monkeys and are reflected in the in vitro metabolism and pharmacokinetics of 1,3-butadiene in these species. This section summarizes the metabolic pathways of 1,3-butadiene disposition and the species differences in 1,3-butadiene pharmacokinetics and metabolism from in vitro and in vivo studies.

3.1.1. Pathways Elucidation

Several in vitro and in vivo studies have elucidated the metabolic pathways of 1,3-butadiene metabolism as shown in Figure 3-1 and summarized in Table 3-1 (Himmelstein et al., 1997). Results from in vitro studies show that 1,3-butadiene undergoes cytochrome P-450-mediated biotransformation to the reactive metabolite epoxybutene, which has also been validated from in vivo studies in rats, mice, and monkeys. Epoxybutene can be activated further to another reactive metabolite, diepoxybutane, or detoxified by epoxide hydrolase to butene diol, as shown by in vitro studies and detected in vivo via their glutathione (GSH) conjugates in rats, mice, hamsters, monkeys, and humans. Further metabolism of these two metabolites can be mediated by either the P-450 system or epoxide hydrolase, giving 1,2-dihydroxy-3,4-epoxybutane. The detoxification of epoxybutene occurs by hydrolysis and GSH conjugation and is mediated by the enzymes epoxide hydrolase and glutathione S-transferase (GST), respectively; these reactions have been supported by both in vitro and in vivo studies. Epoxybutene can also form DNA and hemoglobin (Hb) adducts in both rats and mice. Of greater significance is the identification of crotonaldehyde, a DNA-reactive chemical and known mutagen, as a new product of the oxidative metabolism of butadiene. Crotonaldehyde was formed by the tautomerization of 3-butenal formed by chloroperoxidase-dependent oxidation of 1,3-butadiene and was not a metabolic product of epoxybutene. 3-Butanal rapidly tautomerized to crotonaldehyde at room temperature, which may explain its nondetection in in vitro studies. A possible pathway for the metabolism of 3-butene-1,2-diol, a secondary metabolite of 1,3-butadiene, is oxidative dehydrogenation catalyzed by alcohol dehydrogenase. The production of GSH-epoxide conjugates, *S*-(2-hydroxy-3-buten-1-yl)glutathione (compound I) and *S*-(1-hydroxy-3-buten-2-yl)glutathione (compound II), was confirmed using human placental GST. While compound II is chemically stable, compound I tautomerizes to a stable sulfrane. Because these compounds are of low reactivity (including the stable sulfrane), this biotransformation pathway may represent a physiological protective mechanism against the DNA reactivity of epoxybutene.

3.1.2. Species Differences

3.1.2.1. *In Vitro Metabolism*

Species differences for several reactions described in the previous section are shown by measuring their in vitro reaction rates using microsomal and cytosolic preparations from several organs. Himmelstein et al. (1997) gives a comprehensive summary of the in vitro methodology and the studies that measure the reaction rates of the reactions included in the metabolic pathways shown in Figure 3-1. Table 3-2 summarizes the reaction rates and rate constants obtained from the main studies that compare these differences (modified from Himmelstein et

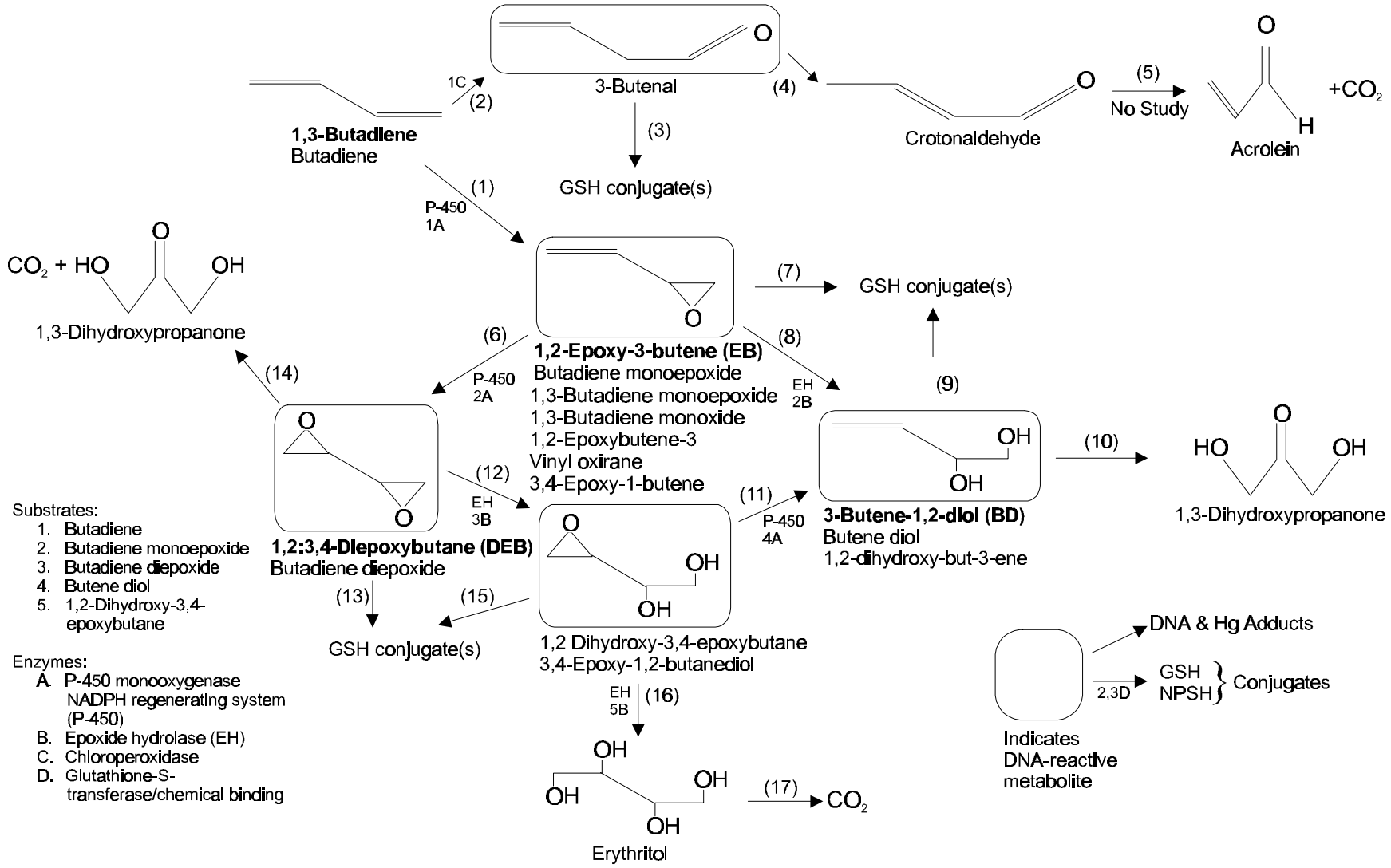
1,3-Butadiene
Butadiene
1C
(2)
3-Butenal
(3)
GSH conjugate(s)
(4)
Crotonaldehyde
(5)
No Study
Acrolein
+CO2
(1)
P-450
1A
CO2 + HO
OH
1,3-Dihydroxypropanone
(6)
P-450
2A
1,2-Epoxy-3-butene (EB)
Butadiene monoepoxide
1,3-Butadiene monoepoxide
1,3-Butadiene monoxide
1,2-Epoxybutene-3
Vinyl oxirane
3,4-Epoxy-1-butene
(7)
GSH conjugate(s)
(8)
EH
2B
(9)
(14)
(12)
EH
3B
1,2:3,4-Diepoxybutane (DEB)
Butadiene diepoxide
(11)
P-450
4A
3-Butene-1,2-diol (BD)
Butene diol
1,2-dihydroxy-but-3-ene
(10)
HO
OH
1,3-Dihydroxypropanone
(13)
(15)
GSH conjugate(s)
1,2 Dihydroxy-3,4-epoxybutane
3,4-Epoxy-1,2-butanediol
EH
5B
(16)
(17)
CO2
Erythritol
Substrates:
1. Butadiene
2. Butadiene monoepoxide
3. Butadiene diepoxide
4. Butene diol
5. 1,2-Dihydroxy-3,4-epoxybutane
Enzymes:
A. P-450 monooxygenase NADPH regenerating system (P-450)
B. Epoxide hydrolase (EH)
C. Chloroperoxidase
D. Glutathione-S-transferase/chemical binding
DNA & Hg Adducts
2,3D
GSH
NPSH
Conjugates
Indicates DNA-reactive metabolite

Figure 3-1. Some pathways in the metabolism of butadiene. Numbers in parentheses represent specific metabolic reactions for which literature references are given in Table 3-1. Reactions (1), (2), (6), and (11) are mediated by cytochrome P-450-dependent monooxygenases. Glutathione (GSH) is a substrate in reactions (3), (7), (9), (13), and (15), which are mediated by glutathione S-transferase or occur spontaneously. A GSH conjugate of reaction(s) is excreted in the urine as *N*-acetyl-*S*-(1-hydroxy-3-butenyl)-L-cysteine. Enzyme-mediated GSH conjugates from reaction (7) include *S*-(2-hydroxy-3-buten-1-yl)glutathione and *S*-(1-hydroxy-3-buten-2-yl)glutathione, which are subsequently excreted in the urine as 1-hydroxy-2-(*N*-acetylcysteinyl)-3-butene and 2-hydroxy-1-(*N*-acetylcysteinyl)-3-butene. The GSH conjugate of reaction (9) is excreted in the urine as 1,2-dihydroxy-4-(*N*-acetylcysteinyl)-butane. A=*S*-(2-hydroxy-3,4-epoxybut-1-yl)glutathione; B=*S*-(1-hydroxy-3,4-epoxybut-2-yl)glutathione; C=*S*-(1,2,3-trihydroxybut-4-yl)glutathione; D=*S*-(1,3,4-trihydroxybut-2-yl)glutathione. The enzyme-mediated or spontaneous formation of GSH conjugates for reaction 13 form A and B, which are excreted in the urine as C and D, respectively. Reactions (10) and (14) are mediated through the pentose phosphate pathway. Reactions (8), (12), and (16) are mediated by epoxide hydrolase or occur spontaneously.

Sources: Dahl et al., 1990; Laib et al., 1990; Elfarra et al., 1991; Himmelstein et al., 1997.

Table 3-1. Metabolic pathways of 1,3-butadiene metabolism

Reaction	Species and tissue	Reference
(1)1,3-Butadiene→EB		
In vitro experiments		
	Wistar rat liver microsomes.	(1a) Malvoisin et al. (1979a)
	SD rat liver microsomes.	(1b) Bolt et al. (1983)
	B6C3F_1 and NMRI mouse; SD and Wistar rat, rhesus monkey, and human (n = 1) postmitochondrial lung and liver fractions.	(1c) Schmidt and Loeser (1985)
	Liver microsomes of rats (strain not stated), mice (strain not stated), and humans (n = 4).	(1d) Wistuba et al. (1989)
	B6C3F_1 mouse liver microsomes.	(1e) Elfarra et al. (1991)
	SD rat, B6C3F_1 mouse, and human (n = 12) liver and lung microsomes.	(1f) Csanády et al. (1992)
	SD rat and B6C3F_1 mouse liver, lung, kidney, and testis microsomes.	(1g) Sharer et al. (1992)
	Purified human myeloperoxidase from human polymorphonuclear leukocytes and B6C3F_1 mouse liver microsomes.	(1h) Duescher and Elfarra (1992)
	Rat (strain not stated) liver microsomes.	(1i) Cheng and Ruth (1993)
	SD rat, B6C3F_1 mouse, and human (n = 6) liver microsomes.	(1j) Duescher and Elfarra (1994)
	B6C3F_1 mouse bone marrow cells and cell lysates, human bone marrow cells, and purified human myeloperoxidase.	(1k) Maniglier-Poulet et al. (1995)

Table 3-1. Metabolic pathways of 1,3-butadiene metabolism (continued)

Reaction	Species and tissue	Reference
In vivo inhalation experiments		
Closed-chamber gas-uptake system	SD rats. EB in exhaled breath.	(1l) Bolt et al. (1983)
	SD rats. Metabolic uptake rate from untreated rats compared with that of rats pretreated with Aroclor 1254 and P-450 inhibitor.	(1m) Bolt et al. (1984)
	$B6C3F_1$ mice. Metabolic uptake rate from untreated rats compared with that of rats pretreated with P-450 inhibitor.	(1n) Kreiling et al. (1986a)
	SD rats and $B6C3F_1$ mice. EB in exhaled breath.	(1o) Kreiling et al. (1987)
	SD rats and $B6C3F_1$ mice, pretreated with 4-methylpyrazole (P-450 inhibitor). Metabolic uptake rate from untreated rats compared with that of rats pretreated with P-450 inhibitor.	(1p) Medinsky et al. (1994)
Nose-only exposure system	SD rats and $B6C3F_1$ mice. EB in blood.	(1q) Bond et al. (1986)
	Cynomolgus monkeys. EB in blood.	(1r) Dahl et al. (1990)
	SD rats and $B6C3F_1$ mice. 1,3-butadiene and EB in blood.	(1s) Himmelstein et al. (1994)
	SD rats and $B6C3F_1$ mice. 1,3-butadiene and EB in blood.	(1t) Bechtold et al. (1995)
	SD rats and $B6C3F_1$ mice. EB in liver and lung tissues.	(1u) Himmelstein et al. (1995)
	SD rats and $B6C3F_1$ mice. EB in blood, fat, heart, liver, lung, spleen, and thymus.	(1v) Thornton-Manning et al. (1995a)
	Female and male SD rats. EB in blood, femur, fat, lung, and mammary tissue.	(1w) Thornton-Manning et al. (1995b)

Table 3-1. Metabolic pathways of 1,3-butadiene metabolism (continued)

Reaction	Species and tissue	Reference
(2) 1,3-Butadiene→3-butenal		
In vitro experiments		
EB and crotonaldehyde in reaction mixture. Proposed 3-butenal as intermediate in formation of crotonaldehyde.	$B6C3F_1$ mouse liver microsomes and purified fungal enzyme, chloroperoxidase.	(2a) Elfarra et al. (1991)
3-Butenal detected at optimal pH of 6.0.	Purified fungal enzyme, chloroperoxidase.	(2b) Duescher and Elfarra (1993)
(3) 3-Butenal→GSH conjugates		
***In vivo inhalation experiments: 3-butenal-GSH conjugate*:**		
N-acetyl-*S*-(1-hydroxy-3-butenyl)-L-cysteine.	SD rats and $B6C3F_1$ mice. Urinary metabolite detected in mouse, not in rat.	(3a) Nauhaus et al. (1996)
(4) 3-Butenal→crotonaldehyde		
In vitro experiments		
	Same experiment as (2a) above.	(4a) Elfarra et al. (1991)
	$B6C3F_1$ mouse and SD rat liver, lung, kidney, and testis microsomes. Crotonaldehyde detected in mouse but not in rat.	(4b) Sharer et al. (1992)
	Same experiment as (1h) above.	(4c) Duescher and Elfarra (1992)
	Same experiment as (1i) above.	(4d) Cheng and Ruth (1993)
Crotonaldehyde identified as tautomerization product of 3-butenal.	Same experiment as (2b) above.	(4e) Duescher and Elfarra (1993)

Table 3-1. Metabolic pathways of 1,3-butadiene metabolism (continued)

Reaction	Species and tissue	Reference
	Human (n = 6), B6C3F$_1$ mouse, and SD rat liver microsomes and microsomes derived from human β-lymphoblastoid cells containing cDNA-expressed CYP 1A1, 1A2, 2A6, 2B6, 2D6, 2D1, 3A4. Same experiment as (1j) above.	(4f) Duescher and Elfarra (1994)
(5) Crotonaldehyde→acrolein + CO_2		No published literature supporting this reaction in vitro or in vivo.
(6) EB→DEB		
In vitro experiments		
	Wistar rat liver microsomes.	(6a) Malvoisin et al. (1979b)
Detected two stereoisomers of DEB.	Wistar rat liver microsomes.	(6b) Malvoisin and Roberfroid (1982)
	SD rat, B6C3F$_1$ mouse, and human liver (n = 12) and lung (n = 5) microsomes. Identified DEB only in mouse liver microsomes.	(6c) Csanády et al. (1992)
	Human microsomes containing cDNA-expressed CYP isozymes (1A1, 1A2, 2A6, 2D6, 2E1, 2F1, 3A4) and SD rat, B6C3F$_1$ mouse and human (n = 10) liver microsomes.	(6d) Seaton et al. (1995)
In vivo inhalation experiments		
	Same experiments as (1s) above. DEB in blood of mice, not rat.	(6e) Himmelstein et al. (1994)
	Same experiments as (1t) above.	(6f) Bechtold et al. (1995)
	Same experiments as (1u) above. DEB in lungs of mice but not rat.	(6g) Himmelstein et al. (1995)

Table 3-1. Metabolic pathways of 1,3-butadiene metabolism (continued)

Reaction	Species and tissue	Reference
In vivo inhalation experiments		
	Same experiments as (1v) above. DEB in blood, heart, and lung, thymus, fat, spleen and liver, higher in mice than in rats.	(6h) Thornton-Manning et al. (1995a)
	Same experiments as (1w) above. DEB in blood, bone marrow, fat, lung, mammary (female only), higher in females than in males.	(6i) Thornton-Manning et al. (1995b)
(7) EB→GSH conjugates		
In vitro experiments		
	Same experiment as (1b) above.	(7a) Bolt et al. (1983)
	SD rat, NMRI mouse, and human (n = 1) liver microsomes and cytosol.	(7b) Kreuzer et al. (1991)
	Purified human placental π class GSH S-transferase.	(7c) Sharer et al. (1991)
	SD rat and B6C3F$_1$ mouse liver, lung, kidney, and testis cytosol. Same experiment as (1g) above.	(7d) Sharer et al. (1992)
	SD rat, B6C3F$_1$ mouse, and human liver (n = 12) and lung (n = 5) microsomes and cytosol. Same experiment as (1f) and (6c) above.	(7e) Csanády et al. (1992)

Table 3-1. Metabolic pathways of 1,3-butadiene metabolism (continued)

Reaction	Species and tissue	Reference
***In vivo experiments*: *EB-GSH conjugates*:**		
1-hydroxy-2-(*N*-acetylcysteinyl)-3-butene and 2-hydroxy-1-(*N*-acetylcysteinyl)-3-butene.	Inhalation exposure of EB to SD and F344/N rats, $B6C3F_1$ mice, Syrian hamsters, and cynomolgus monkeys. Urinary metabolites.	(7f) Sabourin et al. (1992)
S-(2-hydroxy-3-buten-1-yl)glutathione.	i.p. injection of EB to SD rats. Isolated and identified regioisomeric GSH conjugates in bile of rats.	(7g) Sharer and Elfarra (1992)
S-(2-hydroxy-3-buten-1-yl)-*N*-acetyl-L-cysteine and *S*-(1-hydroxy-3-buten-2-yl)-*N*-acetyl-L-cysteine.	SD rats and $B6C3F_1$ mice, by i.p. injection. Urinary metabolites.	(7h) Elfarra et al. (1995)
S-(1-hydroxymethyl)-2-propenyl)-L-cysteine in mouse, not in rat. *N*-acetyl-*S*-(2-(hydroxymethyl)-2-propenyl)-L-cysteine and *N*-acetyl-*S*-(2-hydroxy-3-butenyl)-L-cysteine in both mouse and rats.	Inhalation exposure of rats and mice. Same study as (3a) above. Urinary metabolites.	(7i) Nauhaus et al. (1996)
(8) EB→BD		
In vitro experiments		
	Wistar rat liver microsomes.	(8a) Malvoisin and Roberfroid (1982)
	SD rat liver microsomes treated with inhibitor of epoxide hydrolase. Same experiment as (1b) and (7a) above.	(8b) Bolt et al. (1983)
	SD rat, NMRI mouse, and human (n = 1) liver microsomes.	(8c) Kreuzer et al. (1991)

Table 3-1. Metabolic pathways of 1,3-butadiene metabolism (continued)

Reaction	Species and tissue	Reference
	Rat, mouse, and human lung and liver microsomes. Same study as (1f) and (6c) above.	(8d) Csanády et al. (1992)
	Rat (strain not stated) liver microsomes. Same experiment as (4d) above.	(8e) Cheng and Ruth (1993)
***In vivo inhalation experiments: BD-GSH conjugates*:**		
1,2-dihydroxy-4-(*N*-acetylcysteinyl)-butane. (BD necessary intermediate of this product.)	Same experiment as (7f) above. Urinary metabolites.	(8f) Sabourin et al. (1992)
Human urinary clearance predominantly via epoxide-hydrolase-mediated hydrolysis.	F344/N rats, B6C3F$_1$ mice, and humans in occupational exposure. See (9b) below.	(8g) Bechtold et al. (1994)
	Same study as (3a) and (7i) above.	(8h) Nauhaus et al. (1996)
(9) BD→GSH conjugates		
***In vivo inhalation experiments: BD-GSH conjugates*:**		
1,2-dihydroxy-4-(*N*-acetylcysteinyl)-butane.	Same experiments as (7f) and (8f) above. Urinary metabolites in monkey, rat, hamster, and mouse.	(9a) Sabourin et al. (1992)
1,2-dihydroxy-4-(*N*-acetylcysteinyl)-butane.	Same experiments as (8g) above. Urinary metabolites in humans.	(9b) Bechtold et al. (1994)
N-acetyl-*S*-(3,4-dihydroxybutyl)-L-cysteine in mouse and rat, *N*-acetyl-*S*-(1-(hydroxymethyl)-3,4-dihydroxypropyl)-L-cysteine in mouse but not in rat.	Same study as (3a), (7i), and (8h) above. Urinary metabolites.	(9c) Nauhaus et al. (1996)

Table 3-1. Metabolic pathways of 1,3-butadiene metabolism (continued)

Reaction	Species and tissue	Reference
(10) BD→1,3-dihydroxypropanone		
In vivo inhalation experiments	Same study as (3a), (7i), (8h), and (9c) above.	(10a) Nauhaus et al. (1996)
(11) BD→3,4-epoxy-1,2-butanediol		
In vitro experiments	Wistar rat liver microsomes. Same experiment as (6b) above.	(11a) Malvoisin and Roberfroid (1982)
	Rat (strain not stated) liver microsomes. Same experiments as (4d) and (8e) above.	(11b) Cheng and Ruth (1993)
(12) DEB→3,4-epoxy-1,2-butanediol		
In vitro experiments	Human (n = 6), SD rat, and B6C3F$_1$ mouse liver and lung microsomes.	(12a) Boogaard and Bond (1996)
(13) DEB→GSH conjugates		
***In vitro experiments: DEB-GSH conjugates*:** *S*-(2-hydroxy-3,4-epoxybut-1-yl)glutathione and *S*-(4-hydroxy-2,3-epoxybut-1-yl)glutathione.	*Salmonella typhimurium* TA1535 transfected with rat GSH S-transferase 5-5 cDNA.	(13a) Thier et al. (1995)
	SD rat, B6C3F$_1$ mouse, and human (n = 6) liver cytosol and SD rat and B6C3F$_1$ lung cytosol.	(13b) Boogaard et al. (1996)
***In vivo experiments: DEB-GSH conjugates*:** *N*-acetyl-*S*-(1-(hydroxymethyl)-3,4-dihydroxypropyl)-L-cysteine in mouse, but not in rat.	Same experiment as (3a), (7i), (8h), (9c), and (10a) above. Urinary metabolites.	(13c) Nauhaus et al. (1996)

Table 3-2. Species comparison of reaction rates for epoxidation, GSH conjugation, and hydrolysis reactions involved in the metabolism of 1,3-butadiene

Reaction and tissue	Species	Strain	Reaction rate[a] or V_{max}	K_M (mM)[b]	Reference (exposure concentration)[c]
(1) 1,3-Butadiene→EB					
Liver microsomes	Mouse	All	0.24-9.9		All studies with species differences data
	Rat	All	0.36-45		
	Monkey		----		
	Human		0.121-22.8		
	Mouse	$B6C3F_1$, NMRI	0.24-0.40[d]		(1c) Schmidt and Loeser (1985) (30,000 ppm 1,3-butadiene)
	Rat	SD, Wistar	0.08-0.1[d]		
	Monkey	rhesus	0.73[g]		
	Human		0.12[d]		
	Mouse	$B6C3F_1$	2.6	2.0	(1f) Csanády et al. (1992) (600-25,000 ppm 1,3-butadiene)
	Rat	SD	0.59	3.74	
	Monkey		----		
	Human		1.18	5.14	
	Mouse	$B6C3F_1$	6.4		(1g) Sharer et al. (1992) (330,000-660,000 ppm 1,3-butadiene)
	Rat	SD	3.0		
	Monkey		----		
	Human		----		
	Mouse	$B6C3F_1$	9.2	160	(1j) Duescher and Elfarra (1994) (30,000-660,000 ppm 1,3-butadiene)
	Rat	SD	2.0	120	
	Monkey		----		
	Human		10.4-22.8	200-400	

Table 3-2. In vitro rates and rate constants for epoxidation, GSH conjugation, and hydrolysis reactions involved in the metabolism of 1,3-butadiene (continued)

Reaction and tissue	Species	Strain	Reaction rate[a]	K_M (μM)[b]	Reference (exposure concentration)[c]
Lung microsomes	Mouse	All	2.3-6.1		All studies
	Rat	All	0.16-1.5		
	Human		0.15		
	Mouse	$B6C3F_1$, NMRI	4.4-5.6[e]		(1c) Schmidt and Loeser (1985)
	Rat	SD, Wistar	0.6-0.91[e]		(30,000 ppm 1,3-butadiene)
	Human		----		
	Mouse	$B6C3F_1$	2.3	5.01	(1f) Csanády et al. (1992)
	Rat	SD	0.16	7.75	(600-25,000 ppm 1,3-butadiene)
	Human		0.15	2.0	
	Mouse	$B6C3F_1$	6.1		(1g) Sharer et al. (1992)
	Rat	SD	1.5		(330,000-660,000 ppm 1,3-butadiene)
	Human		----		
Kidney microsomes	Mouse	$B6C3F_1$	23.8		(1g) Sharer et al. (1992)
	Rat	SD	0.5		(330,000-660,000 ppm 1,3-butadiene)
(6) EB → DEB					
Liver microsomes	Mouse	$B6C3F_1$	1.4	141	(6d) Seaton et al. (1995)
	Rat	SD	0.41	145	(5-1,000 μM EB)
	Human		0.38-1.2	304-880	

Table 3-2. In vitro rates and rate constants for epoxidation, GSH conjugation, and hydrolysis reactions involved in the metabolism of 1,3-butadiene (continued)

Reaction and tissue	Species	Strain	Reaction rate[a]	K_M (µM)[b]	Reference (exposure concentration)[c]
(7) EB →GSH conjugates					
Liver cytosol	Mouse	$B6C3F_1$	107	3,100	(7d) Sharer et al. (1992)
	Rat	SD	71	3,100	(2-10 mM EB)
	Human		----		
	Mouse	$B6C3F_1$	500	35,300	(7e) Csanády et al. (1992)
	Rat	SD	241	13,800	(20-200 ppm EB)
	Human		45.1	10,400	
Lung cytosol	Mouse	$B6C3F_1$	12	3,100	(7d) Sharer et al. (1992)
	Rat	SD	3	3,100	(2-10 mM EB)
	Human		----		
	Mouse	$B6C3F_1$	273	36,500	(7e) Csanády et al. (1992)
	Rat	SD	44.2	17,400	(20-200 ppm EB)
	Human		$2.56 \cdot 10^{-4}$ [f]		
Kidney cytosol	Mouse	$B6C3F_1$	16	3,100	(7d) Sharer et al. (1992)
	Rat	SD	7	3,100	(2-10 mM EB)
Testis cytosol	Mouse	$B6C3F_1$	30	3,100	(7d) Sharer et al. (1992)
	Rat	SD	51	3,100	(2-10 mM EB)

Table 3-2. In vitro rates and rate constants for epoxidation, GSH conjugation, and hydrolysis reactions involved in the metabolism of 1,3-butadiene (continued)

Reaction and tissue	Species	Strain	Reaction rate[a]	K_M (µM)[b]	Reference (exposure concentration)[c]
Purified placental GSH S-transferase	Human		500	10,000	(7d) Sharer et al. (1992)
Spontaneous[g]			$2.01 \cdot 10^{-4}$		
(8) EB →BD					
Liver microsomes	Mouse	NMRI	19	1,500	(8c) Kreuzer et al. (1991)
	Rat	SD	17	700	(30, 300, 3,000 ppm EB)
	Human		14	500	
	Mouse	$B6C3F_1$	5.79	1,590	(8d) Csanády et al. (1992)
	Rat	SD	2.48	260	(20-200 ppm EB)
	Human		9.2-58.1	240-1,650	
Lung microsomes[h]	Mouse	$B6C3F_1$	$1.86 \cdot 10^3$		(8d) Csanády et al. (1992)
	Rat	SD	$1.32 \cdot 10^3$		(20-200 ppm EB)
	Human		$3.19\text{-}7.55 \cdot 10^3$		
Spontaneous[g]	Human		$7.75 \cdot 10^{-4}$		(8d) Csanády et al. (1992)
(12) DEB → hydrolysis products					
Liver microsomes	Mouse	$B6C3F_1$	32.0	8,100	(12a) Boogaard and Bond (1996)
	Rat	SD	52.9	2,760	(0.185-15 mM DEB)
	Human		156	4,800	

Table 3-2. In vitro rates and rate constants for epoxidation, GSH conjugation, and hydrolysis reactions involved in the metabolism of 1,3-butadiene (continued)

Reaction and tissue	Species	Strain	Reaction rate[a]	K_M (μM)[b]	Reference (exposure concentration)[c]
Lung microsomes	Mouse		49.3	7,500	(12a) Boogaard and Bond (1996)
	Rat		19.3	7,100	(0.558-5 mM EB)
	Human		21.7	2,830	
(13) DEB → GSH conjugates					
Liver cytosol	Mouse	$B6C3F_1$	162	6,400	(13b) Boogaard et al. (1996)
	Rat	SD	186	24,000	(0.1-25 mM DEB)
	Human		6.4	2,100	
Lung cytosol	Mouse		38.5	1,700	(13b) Boogaard et al. (1996)
	Rat		17.1	4,200	(0.1-25 mM DEB)
Spontaneous[g]			$1.65 \cdot 10^{-3}$		(13b) Boogaard et al. (1996) (0.1-25 mM DEB)

[a]Reaction rates are either from the reported reaction rates (units of nmol · min^{-1} · mg microsomal or cytosolic $protein^{-1}$ unless otherwise noted) or from maximum reaction rate (V_{max} in units of nmol · min^{-1} · mg microsomal or cytosolic $protein^{-1}$ unless otherwise noted), when corresponding K_M is given.
[b] Concentration at one-half maximum reaction rate.
[c] Numbers and letters in parentheses preceding reference refer to appropriate metabolic pathway shown in Figure 3-1 and summarized in Table 3-1.
[d] Values reported were corrected from nmol · min^{-1} · g $tissue^{-1}$ to nmol · min^{-1} · mg microsomal $protein^{-1}$ assuming values of 11.6 (mouse), 16.8 (rat), and 14.5 (human) mg microsomal protein · g $tissue^{-1}$ reported by Csanády et al. (1992).
[e] Value has units of nmol·min^{-1} · g $tissue^{-1}$.
[f] None of the human lung cytosolic fractions displayed Michaelis-Menten reaction kinetics. The reactions are described by first-order k_{gsh} with units of L · nmol · min^{-1} · mg $protein^{-1}$.
[g] Spontaneous conjugation rate of EB or DEB with GSH is described by first-order k_{gsh} with units L · min^{-1} · $mmol^{-1}$.
[h] Hydrolysis of epoxybutene in lung microsomes is described as first-order k_{hy} with units of min^{-1} · mg $protein^{-1}$.
[I] Spontaneous hydrolysis rate of epoxybutene or diepoxybutane in 0.1 M phosphate buffer is described as first-order k_{hy} with units of min^{-1}.

Source: Modified from Himmelstein et al., 1997.

al., 1997). In general, the range of reaction rates or maximal reaction velocity (V_{max}) for different reactions in different tissues do not provide a clear pattern of species differences; in particular, the range of values for human tissues spans the range of values for both rats and mice. However, within any single study, for the oxidation of 1,3-butadiene to epoxybutene, the reaction rates of liver and lung microsomes are higher in rats than in mice. Multiple cytochrome P-450 enzymes are involved in the metabolism of 1,3-butadiene. For example, in human liver microsomes, the metabolic oxidation of 1,3-butadiene to epoxybutene is principally mediated by P-450 isoenzymes 2A6 and 2E1. Biotransformation of 1,3-butadiene to the non-DNA-reactive butene diol is the predominant pathway observed in in vitro metabolism studies that used hepatic microsomes from rats and humans, and formation of the DNA-reactive diepoxybutane is relatively minor in these species. However, the latter pathway is significant in mouse hepatic microsomes. 1,3-Butadiene can also be metabolized to epoxybutene by human myeloperoxidase and by mouse and human bone marrow cells.

In the Csanády et al. (1992) study, the authors also extrapolated the kinetic constants obtained from in vitro experiments to equivalent in vivo rates by adjusting the in situ protein content and organ weights across species, as shown in Table 3-3. However, for GST, Kohn and Melnick (1993) pointed out that the rate constants should be adjusted to the mg cytosolic protein/g liver instead of to the mg microsomal protein/g liver as done by Csanády et al. (1992). The corrected values are also included in Table 3-3. These can all be used in pharmacokinetic models as hepatic and lung metabolic clearance.

3.1.2.2. *In Vivo Pharmacokinetics*

In vivo pharmacokinetic studies examine absorption, distribution, metabolism, and/or elimination. Most studies report results on several of these four components. Absorption is often measured either by the distribution of 1,3-butadiene and/or its metabolites in tissue organs or by the elimination of 1,3-butadiene metabolites in excreted urine, feces, and exhaled air. In vivo metabolism studies include measurements of concentration profiles of the various metabolite pools after exposure to butadiene. Metabolic kinetic constants are usually calculated from the rate of formation of the metabolites or from the clearance rate evaluated from excretion data. This section summarizes the in vivo pharmacokinetic studies. Because inhalation is the principal route of exposure to 1,3-butadiene, most of the absorption data for the chemical have been derived from inhalation exposure studies. Based on the blood:air partition coefficient for 1,3-butadiene (0.603 in vitro; 0.645 in vivo), the passage of 1,3-butadiene from the air into the blood is by simple diffusion (Carpenter et al., 1944).

Table 3-3. Rate constants for in vivo hepatic clearance of 1,3-butadiene and EB[a] (extrapolated from in vitro)

	Cytochrome P-450 monooxygenase[b]	Epoxide hydrolase[b,c]	Cytosolic glutathione S-transferase[b]	Microsomal conjugation of EB with GSH[d]	First-order hydrolysis
Mouse	55.9 0.55[e]	0.16	4.4	0.011	0.0028
Rat	7.92	0.48	5.7	0.024	0.0022
Human	6.19	0.86	0.46	0.069	0.0014

[a]Values are in units of L/h/kg.
[b]In vivo V_{max} values were calculated from in vitro V_{max} (tables 3-1 through 3-3) and adjusted for interspecies differences in microsomal and cytosolic protein concentrations and liver volume. Mouse, rat, and human liver microsomal concentrations were 11.6, 16.8, and 14.5 mg/g liver, respectively. Mouse, rat, and human liver cytosolic concentrations were 11.6, 16.8, and 14.5 mg/g liver, respectively. Mouse, rat, and human liver cytosolic concentrations were 82.8, 108, and 58 mg/g liver, respectively. Liver organ volumes for mice, rats, and humans were 6.2, 5.0, and 3.1% of body weight, respectively. In vivo hepatic clearance values (V_{max}/K_M expressed in L/h/kg) were estimated by dividing the in vivo V_{max} values by the apparent in vitro K_M's for the reaction.
[c]Modified according to Kohn and Melnick, 1993.
[d]For nonenzymic hydrolysis and reaction with glutathione, in vivo clearance was calculated using the organ fractions in footnote b. To estimate the in vivo clearance for reaction with glutathione, a concentration of 10 mM GSH was used.
[e]Rate constant for metabolism of EB to DEB.

Sources: Csanády et al., 1992; Kohn and Melnick, 1993.

Two main in vivo inhalation systems are used to conduct inhalation studies. The first one is the closed-system inhalation chamber, and the second one is the nose-only exposure inhalation system. These studies are reviewed by Himmelstein et al. (1997) and summarized in Tables 3-4 to 3-6 for the closed inhalation chamber studies and Tables 3-7 to 3-10 for the nose-only inhalation studies.

In the closed-system inhalation chamber study, rats or mice are placed in a desiccator jar chamber. Two rats or up to eight mice per experiment are exposed to different initial 1,3-butadiene chamber concentrations. Air samples from the desiccator are measured directly by gas chromatography-mass spectrometry (GC-MS) through an air valve. With the use of a two-compartment pharmacokinetic model (Filser and Bolt, 1981), shown in Figure 3-2, uptake and clearance kinetic constants of 1,3-butadiene and epoxybutene can be evaluated, as shown in Tables 3-5 and 3-6, which give the results of these studies. Because the metabolic elimination rate constant (k_{el}) cannot be determined accurately from the gas uptake studies, 1,3-butadiene and epoxybutene were administered intraperitoneally to the mice and rats, and exhaled 1,3-butadiene and epoxybutene concentrations were monitored in the chamber and used to evaluate k_{el} (Bolt et al., 1984). Tables 3-5 and 3-6 show that for both 1,3-butadiene and epoxybutene, uptake ($k_{12}V_1$) and clearance (Cl_{tot}) in mice are about twofold greater than in rats. Although the exhalation rate constant (k_{21}) and metabolic elimination rate constant (k_{el}) are comparable for 1,3-butadiene in both mice and rats, mice exhaled epoxybutene about twice as much as rats (k_{21}), whereas the metabolic rate constant (k_{el}) is about fivefold higher in rats than in mice (Laib et al., 1990). Under these conditions, the steady-state epoxybutene concentration in mice is about sixfold that in rats (Melnick and Huff, 1992; Himmelstein et al., 1994).

A second inhalation experimental system is the nose-only exposure, where exhaled breath is sampled by placing the animals in plethysmography tubes. Additional blood and tissue samples can also be obtained by sacrifice of the animals after different exposure durations. However, while the air samples are measured at real time, all blood and tissue samples are subjected to some time delay due to processing of the samples. These studies are summarized in Table 3-7 (modified from Himmelstein et al., 1997). Table 3-8 summarizes the results of the studies showing that 1,3-butadiene and its epoxide metabolites (epoxybutene and diepoxybutane) have been found in blood at different inhalation exposure concentrations to 1,3-butadiene in rats, mice, and monkeys.

Thornton-Manning et al. (1995a) also examined the disposition of epoxybutene and diepoxybutane in various tissues following nose-only inhalation exposure of male Sprague-

Table 3-4. Summary of closed-chamber inhalation studies

Reaction and reference[a]	Description of experiment	Finding
(1) 1,3-Butadiene→EB		
(1l) Bolt et al. (1983)	Exposure of SD rats to butadiene at 6,000 to 7,000 ppm initial concentration.	Quantified epoxybutene in exhaled breath. Peak concentrations of epoxybutene were 2 to 4 ppm at 15 h after exposure.
(1m) Bolt et al. (1984)	Exposure of SD rats to butadiene at initial concentrations ranged from 90 to 12,000 ppm.	Metabolic uptake rate = 220 μmol · h^{-1} · kg^{-1} for untreated rats when the butadiene chamber concentration was >1,500 ppm. Pretreatment with Aroclor 1254 caused a linear increase in the metabolic uptake rate from 220 to 1,200 μmol · h^{-1} · kg^{-1}; P-450 inhibitor diethyldithiocarbamate completely inhibited metabolism.
(1n) Kreiling et al. (1986a)	Exposure of $B6C3F_1$ mice to butadiene at initial concentrations ranged from 10 to 5,000 ppm.	Maximal metabolic uptake rate = 400 μmol · h^{-1} · kg^{-1}. Pretreatment of mice with P-450 inhibitor diethyldithiocarbamate completely inhibited uptake of butadiene.
Filser and Bolt (1984)	Exposure of SD rats to epoxybutene at initial concentration ranged from 500 to 5,000 ppm (not reported by author, estimated from Figure 3-2 in their paper).	Linear metabolic uptake occurred up to 5,000 ppm. V_{max} > 2,600 umol · h^{-1} · kg^{-1} (see text for description of model used in calculation).

Table 3-4. Summary of closed-chamber inhalation studies (continued)

Reaction and reference[a]	Description of experiment	Finding
Uptake of EB[b]		
Kreiling et al. (1987)	Exposure of B6C3F$_1$ mice to epoxybutene at initial concentration ranging from 10 to 5,000 ppm (estimated from Figure 1 of Kreiling et al., 1986b).	Saturated metabolic uptake occurred between 100 and 500 ppm. V_{max} = 350 umol $\cdot h^{-1} \cdot kg^{-1}$.

Abbreviations: ppm = parts per million; SD = Sprague-Dawley rat; V_{max} = Michaelis-Menten enzyme kinetic constant expressing maximum metabolic rate.

[a] Numbers and letters preceding reference refer to the appropriate metabolic pathway shown in Figure 3-1 and summarized in Table 3-1.
[b] Reaction rates measured as uptake of epoxybutene, which could involve several reactions shown in Figure 3-1 and summarized in Table 3-1.

Source: Modified from Himmelstein et al., 1997.

Table 3-5. Toxicokinetic parameters for uptake and elimination of 1,3-butadiene in mice and rats

Parameter (units)	Mouse	Rat	Definition of parameter
$K_{12}V_1$ (mL/h)	10,280	5,750	Equilibrium constant between chamber volume and test animals; first order, $V_1 \rightarrow V_2$.
K_{21} (h^{-1})	3.2	2.5	Equilibrium rate constant between chamber volume and animals; first order, $V_2 \rightarrow V_1$.
K_{eq} (NA)	2.7	2.3	Static equilibrium constant representing virtual absence of metabolism.
K_{st} (NA)	1	0.5	Steady-state concentration; ratio of concentration in animal to chamber concentration.
k_{el} (h^{-1})	7.6	8.8	First-order metabolic elimination rate constant.
Cl_{tot}[a,b] (mL/h)	7,300	4,500	Total clearance of chemical from chamber.
V_{max} (µmol/h/kg)	400	220	Maximum rate of metabolism of chemical.

[a]Calculated for $V_1 \rightarrow \infty$.
[b]Valid for linear range of metabolism (up to 1,000 ppm for both species).
NA = not applicable.
Source: Filser and Bolt, 1981; Kreiling et al., 1990.

Table 3-6. Toxicokinetic parameters for the uptake and elimination of epoxybutene in rats and mice

Parameter (units)	Mouse	Rat	Definition of parameter
$k_{12}V_1$ (mL/h)	33,500	13,800	Equilibrium constant between chamber volume and test animals; first order, $V_1 \rightarrow V_2$.
K_{21} (h^{-1})	0.79	0.37	Equilibrium rate constant between chamber volume and animals; first order, $V_2 \rightarrow V_1$.
K_{eq} (NA)	42.5	37	Static equilibrium constant representing virtual absence of metabolism.
K_{st} (NA)	10.2	1.16	Steady-state concentration; ratio of concentration in animal to chamber concentration.
k_{el} (h^{-1})	2.3	11.5	First-order metabolic elimination rate constant.
Cl_{tot}[a,b] (mL/hr)	24,900	13,400	Total clearance of chemical from chamber.
V_{max} (µmol/h/kg)	350	>2,600	Maximum rate of metabolism of chemical.
Metabolic saturation (ppm)	500	>5,000	Concentration resulting in saturated metabolism.

[a]Calculated for $V_1 \rightarrow \infty$.
[b]Valid for linear range of metabolism (up to 1,000 ppm for both species).
NA = not applicable.
Source: Filser and Bolt, 1981; Kreiling et al., 1987; Laib et al., 1990.

Table 3-7. Summary of nose-only inhalation studies

Reaction and reference[a]	Description of experiment	Finding
(1) 1,3-Butadiene→EB		
(1q) Bond et al. (1986)	Exposure of SD rats and B6C3F$_1$ mice to 1,3-butadiene (7, 70, or 1,000 ppm for up to 6 h).	Epoxybutene tentatively identified in blood after 2, 4, or 6 h of exposure. EB blood concentrations were 0.4 and 3.3 µM in rats exposed to 70 and 1,000 ppm 1,3-butadiene and 0.5, 1.6, and 13 µM in mice exposed to 7, 70, and 1,000 ppm, respectively. Concentration of EB in blood of mice 2 to 3 times > rats.
(1r) Dahl et al. (1990)	Exposure of cynomolgus monkeys to 1,3-butadiene (10, 300, or 8,000 ppm for 2 h).	Blood samples collected at single time immediately after exposure. Quantitation of EB in blood by vacuum-trap distillation method. Results shown in Table 3-8. Total butadiene metabolites in blood were 5 to 50 times lower in monkey than in the mouse and 4 to 14 times lower than in the rat (rat and mouse data were from Bond et al., 1986).
(1s) Himmelstein et al. (1994)	Exposure of SD rats and B6C3F$_1$ mice to 1,3-butadiene (62.5, 625, or 1,250 ppm up to 6 h; blood collected at 2 to 6 h of exposure and up to 30 min postexposure).	1,3-Butadiene and EB pharmacokinetics characterized in blood by GC (butadiene) and GC-MS (EB). Butadiene steady-state concentrations (µM) ranged from 2.4 to 58 (mice) and 1.3 to 37 (rats). EB steady-state concentrations (µM) ranged from 0.56 to 8.6 (mice) and 0.07 to 1.3 (rats); EB blood concentration in mice was 4 to 8 times > rats.
(1t) Bechtold et al. (1995)	Exposure of SD rats and B6C3F$_1$ mice to 1,3-butadiene (100 ppm for 4 h; blood collected at end of exposure).	Quantitated butadiene and EB in blood by GC-GC-MS. Blood levels (µM) of butadiene were 4.1 (rat) and 2.9 (mouse). Blood levels of EB (µM) were 0.10 (rat) and 0.38 (mouse).
(1u) Himmelstein et al. (1995)	Exposure of SD rats and B6C3F$_1$ mice to 1,3-butadiene (625, 1,250, or 8,000 [rats only] ppm for 6 h; tissue samples collected at 3 and 6 h of exposure and 6 and 12 min postexposure).	Quantitated EB concentration by GC-MS in mouse lung 14 times > rat lung, mouse liver 5 to 8 times > rat liver. Peak concentrations of EB (nmol · g tissue^{-1}) during exposures were 2.6 to 3.7 (mouse lung), 0.16 to 1.3 (rat lung), 0.58 to 0.93 (mouse liver), and 0.06 to 1.2 (rat liver).

Table 3-7. Summary of nose-only inhalation studies (continued)

Reaction and reference[a]	Description of experiment	Finding
(1v) Thornton-Manning et al. (1995a)	Exposure of SD rats and B6C3F$_1$ mice to 1,3-butadiene (62.5 ppm for 4 h; samples collected at 2 and 4 h of exposure and at 0.5 and 1 h postexposure included blood, fat, heart, liver, lung, spleen, and thymus).	Quantitated EB by GC-GC-MS. Detection limits were 0.031, 0.037, and 0.062 nmol · g tissue^{-1} in blood, heart, and lung, respectively. EB concentrations were 3 to 74 times higher in tissues of mice compared with rats. EB was not detected in lung or liver of rats.
(1w) Thornton-Manning et al. (1995b)	Exposure of female and male SD rats to butadiene (6.25 ppm for 6 h, samples collected at end of exposure included blood, femur, fat, lung, and mammary tissue).	Quantitated EB by GC-GC-MS. EB concentrations were similar for males and females. At 6 h exposure. Results summarized in Table 3-10.
(1y) Thornton-Manning et al. (1996)	Exposure of female SD rats and female B6C3F$_1$ mice after either a single 6 h (daily 6 h exposure for 10 days to 62.5 ppm 1,3-butadiene).	Quantitated EB by GC-GC-MS. EB levels were 5- and 1.6-fold higher in mammary tissue and 2- and 1.4-fold higher in fat tissue in rats and mice, respectively, after repeated exposures. DEB levels were 7.7 ± 2.2 and 12.5 ± 0.8 pmol/g in fat of rats and 265 ± 19 and 191 ± 29 pmol/g in mammary tissue of mice after single and repeated inhalation exposures, respectively.
(3) 3-Butenal→GSH conjugates		
(3a) Nauhaus et al. (1996)	Exposure of SD rats and B6C3F$_1$ mice to 1,3-[^{13}C]-butadiene (800 ppm up to 5 h; urine collected during exposure and for up to 20 h postexposure).	Quantitated the urinary metabolite *N*-acetyl-*S*-(1-hydroxy-3-butenyl)-L-cysteine in mouse urine using ^{13}C-NMR. This metabolite represented 3.7% of total urinary metabolites excreted by mice but was not detected in rat urine.
(6) EB→DEB		
(6e) Himmelstein et al. (1994)	Exposure of rats and mice to 1,3-butadiene. Same conditions as (1s) above.	Quantitated time course of DEB in blood of mice by GC-MS. Peak concentrations of DEB in the blood of mice were 0.65, 1.9, and 2.5 µM after 6 h of exposure to 62.5, 625, or 1,250 ppm 1,3-butadiene; DEB not quantitated in rats. Detection limit = 0.13 µM.

Table 3-7. Summary of nose-only inhalation studies (continued)

Reaction and reference[a]	Description of experiment	Finding
(6f) Bechtold et al. (1995)	Exposure of rats and mice to 1,3-butadiene. Same conditions as (1t) above.	Same finding as (6d) above. DEB detected in mouse and not rat blood; DEB concentration = 0.33 µM in mouse blood after 4 h exposure to 100 ppm 1,3-butadiene. Detection limit = 0.1 µM.
(6g) Himmelstein et al. (1995)	Exposure of rats and mice to 1,3-butadiene. Same conditions as (1u) above.	Quantitated time course of DEB in lungs of mice by GC-MS. Peak concentrations in mice exposed to 625 and 1,250 ppm 1,3-butadiene were 0.71 and 1.5 nmol · g tissue^{-1}, respectively. Detection limit = 0.04 µM.
(6h) Thornton-Manning et al. (1995a)	Exposure of rats and mice to 1,3-butadiene. Same conditions as (1v) above.	Quantitated DEB by GC-GC-MS. Detection limits were 0.0016, 0.031, and 0.026 nmol · g tissue^{-1} in blood, heart, and lung, respectively. DEB concentrations were 40- to 163-fold lower in rat tissues compared with mice. DEB was not detected in liver of rats. Results summarized in Table 3-9.
(6i) Thornton-Manning et al. (1995b)	Exposure of female and male SD rats to 1,3-butadiene. Same conditions as (1w) above.	Quantitated DEB by GC-GC-MS. DEB concentrations were 3.6- to 7.1-fold greater in tissues of female rats compared with tissues of male rats. Results summarized in Table 3-9.
(7) EB→GSH conjugates		
(7f) Sabourin et al. (1992)	Exposure of SD and F344/N rats, B6C3F$_1$ mice, Syrian hamsters, and cynomolgus monkeys to 1,3-[^{14}C]-butadiene (8,000 ppm, 0.78 µCi/mmol).	Products identified in urine included 1-hydroxy-2-(*N*-acetylcysteinyl)-3-butene and 2-hydroxy-1-(*N*-acetylcysteinyl)-3-butene.
(7i) Nauhaus et al. (1996)	Exposure of rats and mice to 1,3-butadiene. Same study as (3a) above.	Quantitated urinary metabolites (as percentage of total ^{13}C) using NMR. *S*-(1-(hydroxymethyl)-2-propenyl)-L-cysteine (4.7%) present in mouse urine but not detected in rat urine. *N*-acetyl-*S*-(2-(hydroxymethyl)-2-propenyl)-L-cysteine was present in mouse (22%) and rat (53%) urine. *N*-acetyl-*S*-(2-hydroxy-3-butenyl)-L-cysteine was present in mouse (44%) and rat (18%) urine.

Table 3-7. Summary of nose-only inhalation studies (continued)

Reaction and reference[a]	Description of experiment	Finding
(8) EB→BD		
(8g) Bechtold et al. (1994)	Exposure of F344/N rats and B6C3F $_1$ mice to 1,3-butadiene (11.7 ppm for 4 h by nose-only exposure). In vivo inhalation exposure of humans occupationally exposed to 1,3-butadiene (human study described in more details in text).	Predominant pathway for clearance of epoxybutene in humans is by epoxide hydrolase-mediated hydrolysis rather than direct conjugation with GSH. See (9b) below.
(8h) Nauhaus et al. (1996)	Exposure of rats and mice to 1,3-butadiene. Same study as (3a) and (7i) above.	Quantitated BD in mouse and rat urine using ^{13}C-NMR. This metabolite represented 2.9% and 5.0% of total ^{13}C-metabolites excreted by mice and rats, respectively.
(9) 3-Butene→BD		
(9b) Bechtold et al. (1994)	Same experiment as (8g) above.	Quantitated 1,2-dihydroxy-4-(*N*-acetylcysteinyl)-butane in human urine using isotope-dilution GC-MS. Metabolite represents 97% of GSH conjugates derived directly from EB (see (7g) above) or indirectly by epoxide hydrolase-mediated hydrolysis and GSH conjugation of BD (see (8e) above).
(9c) Nauhaus et al. (1996)	Exposure of rats and mice to 1,3-butadiene. Same study as (3a), (7i), and (8h) above.	Quantitated *N*-acetyl-*S*-(3,4-dihydroxybutyl)-L-cysteine in mouse (7.1%) and rat (26.4%) urine using ^{13}C-NMR. *N*-acetyl-*S*-(1-(hydroxymethyl)-3,4-dihydroxypropyl)-L-cysteine occurred in mouse (7.1%) urine but was not detected in rat urine. This latter metabolite was also surmised to be a product of DEB as described in reaction 13 below.

Table 3-7. Summary of nose-only inhalation studies (continued)

Reaction and reference[a]	Description of experiment	Finding
(10) BD→1,3-dihydroxypropanone		
(10a) Nauhaus et al. (1996)	Exposure of rats and mice to 1,3-butadiene. Same study as (3a), (7i), (8h), and (9c) above.	Quantitated 1,3-dihydroxypropanone in rat urine as 5.3% of total ^{13}C-metabolites excreted. It was not detected in mouse urine. Transformation of DEB to 1,3-dihydroxypropanone may also contribute to excretion of this compound (see reaction 14 below). This reaction would also be expected to contribute to the formation of CO_2.
(13) DEB→GSH conjugates		
(13c) Nauhaus et al. (1996)	Exposure of rats and mice to 1,3-butadiene. Same study as (3a), (7i), (8h), (9c), and (10a) above.	Quantitated *N*-acetyl-*S*-(1-hydroxymethyl)-3,4-dihydroxypropyl)-L-cysteine in mouse (7.1%) urine. This metabolite was not detected in rat urine and also may be formed as a product of BD as described in study (9c) above.
(14) DEB→1,3-dihydroxypropanone		
(14a) Nauhaus et al. (1996)	Exposure of rats and mice to 1,3-butadiene. Same study as (3a), (7i), (8h), (9c), (10a), and (13c) above.	Quantitated 1,3-dihydroxypropanone in rat urine as 5.3% of total ^{13}C-metabolites excreted. It was not detected in mouse urine. Transformation of BD to 1,3-dihydroxypropanone may also contribute to excretion of this compound (see study (10a) above). This reaction would also be expected to contribute to the formation of CO_2.
(15) 3,4-Epoxy-1,2-butanediol→GSH conjugates	No published literature supporting this reaction.	GSH conjugates of epoxybutanediol have not been quantitated in either in vitro or in vivo studies.

Table 3-7. Summary of nose-only inhalation studies (continued)

Reaction and reference[a]	Description of experiment	Finding
(17) Erythritol→CO_2	No published literature supporting this reaction.	Bond et al. (1986) showed that CO_2 was exhaled in the breath of SD rats and B6C3F$_1$ mice exposed to 1,3-[^{14}C]-butadiene, although the chemical reactions leading to CO_2 formation are unknown. Mass balance of $^{14}CO_2$ from exhaled breath and residual $^{14}CO_2$ from carcass showed that mice had greater uptake of butadiene than rats. Assumption is made that CO_2 derives from erythritol. Dahl et al. (1991) also measured $^{14}CO_2$ in exhaled breath of monkeys exposed to 1,3-[^{14}C]-butadiene. Uptake and retention of ^{14}C in mice > rat > monkey.

Abbreviations: BD = 3-butene-1,2-diol; DEB = 1,2:3,4-diepoxybutane; CO_2 = carbon dioxide; EB = 1,2-epoxy-3-butene; GC = gas chromatography; GSH = glutathione; MS = mass spectrometry; NMR = nuclear magnetic resonance spectrometry; ppm = parts per million; SD = Sprague-Dawley rat.

[a] Numbers and letters preceding reference refer to the appropriate metabolic pathway shown in Figure 3-1 and summarized in Table 3-1.

Source: Modified from Himmelstein et al., 1997.

Table 3-8. Comparison of 1,3-butadiene, epoxybutene, and diepoxybutane blood concentration data from different species of laboratory animals exposed to 1,3-butadiene by inhalation

Species	Butadiene exposure concentration (ppm) Mean (SE)[a]	Concentration of analyte in blood (nM analyte · ppm^{-1}) Mean (SE) 1,3-Butadiene	EB	DEB	Reference
Mouse	7.83 (0.02)	72 (20)	68 (9)	8.5 (0.8)	Bond et al. (1986)[b]
	80 (0.2)	5.4 (0.6)	20 (2)	2.1 (0.2)	Bond et al. (1986)
	1,031 (13)	5.8 (0.6)	12.9 (0.5)	2.3 (0.3)	Bond et al. (1986)
	71 (7)	34 (4)	7.9 (1)	9.2 (1.7)	Himmelstein et al. (1994)[c]
	603 (44)	61 (7)	6.1 (0.8)	3.2 (0.4)	Himmelstein et al. (1994)
	1,282 (33)	45 (3)	6.7 (0.5)	2.0 (0.4)	Himmelstein et al. (1994)
	101 (4)	29 (5)	3.8 (0.6)		Bechtold et al. (1995)[d]
	93 (5)			3.5 (0.9)	
Rat	73.9 (0.4)	1.8 (0.2)	5.4 (0.4)	1.1 (0.2)	Bond et al. (1986)[b]
	949 (12)	3.2 (0.2)	3.5 (0.4)	0.8 (0.1)	Bond et al. (1986)
	63 (2)	21 (1)	1.1 (0.2)		Himmelstein et al. (1994)[c,e]
	616 (8)	29 (1)	1.5 (0.1)		Himmelstein et al. (1994)

Table 3-8. Comparison of 1,3-butadiene, epoxybutene, and diepoxybutane blood concentration data from different species of laboratory animals exposed to 1,3-butadiene by inhalation

	Butadiene exposure concentration (ppm)	Concentration of analyte in blood (nM analyte · ppm^{-1})			
		Mean (SE)[a]			
Species	Mean (SE)	Butadiene	EB	DEB	Reference
Rat	1,249 (3)	30 (1)	1 (0.1)		Himmelstein et al. (1994)
	7,938[f]	32 (1)	0.18 (0.01)		Himmelstein et al. (1995)
	97 (2)	42 (4)	0.6 (0.1)		Bechtold et al. (1995)[d]
Monkey	10.1 (0.1)	0.8 (0.4)	0.16 (0.05)	0.19 (0.06)	Dahl et al. (1990)[f] for EB
	310 (10)	1.8 (1.3)	1.6 (0.9)	0.9 (0.5)	Dahl et al. (1991)[f,j] for DEB
	7,760 (170)	4.1 (0.5)	0.14 (0.06)	0.08 (0.03)	

[a] Standard error (SE) combines variation of butadiene exposure concentration and blood concentration data.
[b] Pooled mean ± SE of samples (n = 9) collected after 2, 4, and 6 h of exposure; animals removed from 1,3-butadiene exposure and exhaled 1,3-butadiene before blood was collected; ^{14}C-labeled analytes were recovered by vacuum-line cryogenic distillation and quantitated by liquid scintillation counting.
[c] Values are means ± SE (n = 6-33 samples) for blood collected between 2 and 6 h of exposure; animals continued to inhale 1,3-butadiene as blood was collected; 1,3-butadiene was quantitated by a vial headspace equilibrium technique using GC-flame ionization detection; detection limit = 0.3 µM.
[d] Values are means ± SE (n = 6) for samples collected at 4 h of exposure; animals continued to inhale 1,3-butadiene as blood was collected; analytes were recovered by vacuum-line cryogenic distillation and analyzed by GC-GC-MS; detection limit = 0.1 µM.
[e] One exposure was conducted at this 1,3- butadiene concentration, therefore no SE reported.
[f] Blood (n = 3) collected immediately following 2 h exposure using indwelling catheter; 1,3-butadiene recovered by vacuum-line cryogenic distillation and quantitated as ^{14}C-labeled equivalent using liquid scintillation counting.
[g] EB and DEB were recovered by extraction into methylene chloride and quantitated by GC-MS; detection limits = 0.03 µM for EB and 0.13 µM for DEB.
[h] Detection limits for EB and DEB = 0.02 µM and 0.01 µM, respectively.
[i] The authors exposed the monkeys to 1,3-[^{4}C]-butadiene and vacuum-line cryogenic distillation for this compound includes ^{14}C-labeled DEB, BD, and potentially other unidentified ^{14}C-metabolites. Since this study looked at products resulting from several reactions, it was not included in Table 3-7 (which described single reactions in the pathways).

Source: Modified from Himmelstein et al., 1997.

Table 3-9. Tissue levels of epoxybutene and diepoxybutane (pmol/g tissue) in male rats and male mice exposed by inhalation to 62.5 ppm 1,3-butadiene for 4 h

Tissue[a]	EB		DEB[a]	
	Rats	Mice	Rats	Mice
Blood	36 ± 7	295 ± 27	5 ± 1	204 ± 15
Heart	40 ± 16	120 ± 15	3 ± 0.4	144 ± 16
Lung	ND[b]	33 ± 9	0.7 ± 0.2[c]	114 ± 37
Liver	ND	8 ± 4	ND	20 ± 4
Fat	267 ± 14	1,302 ± 213	2.6 ± 0.4	98 ± 15
Spleen	7 ± 6	40 ± 19	1.7 ± 0.5[c]	95 ± 12
Thymus	12.5 ± 3.2	104 ± 55	2.7 ± 0.7[c]	109 ± 19
Bone marrow[d]	0.2 ± 0.1	2.3 ± 1.5	ND	1.4 ± 0.3

[a]Mean ± SE; n = 3 or 4.
[b]ND = not detected; indicates that analyte was not detected or was not above control level.
[c]Includes at least one ND value.
[d]As mean pmol/mg protein ± SE.

Source: Modified from Thornton-Manning et al., 1995a.

Table 3-10. Tissue levels of epoxybutene and diepoxybutane (pmol/g tissue) in male and female rats exposed by inhalation to 62.5 ppm 1,3-butadiene for 6 h

Tissue[a]	EB		DEB	
	Males	Females	Males	Females
Blood	25.9 ± 2.9	29.4 ± 2.0	2.4 ± 0.4	11.4 ± 1.7[c]
Femur	9.7, 9.3	10.4 ± 1.0	1.1, 1.8	7.1 ± 1.3[c]
Lung	12.7 ± 5.0	2.7 ± 4.3	1.4 ± 0.8[b]	4.8 ± 0.7[c]
Fat	175 ± 21	203 ± 13	1.1 ± 0.1	7.7 ± 1.3[c]
Mammary	ND	57.4 ± 4	ND	10.5 ± 2.4[c]

[a]n = 3, except for male femur, where n = 2.
[b]One value was not detectable; instrument detection limit/2 was substituted to calculate the mean.
[c]Statistically greater than male tissue value, $p \leq 0.05$.

ND = not determined.
Source: Modified from Thornton-Manning et al., 1995b.

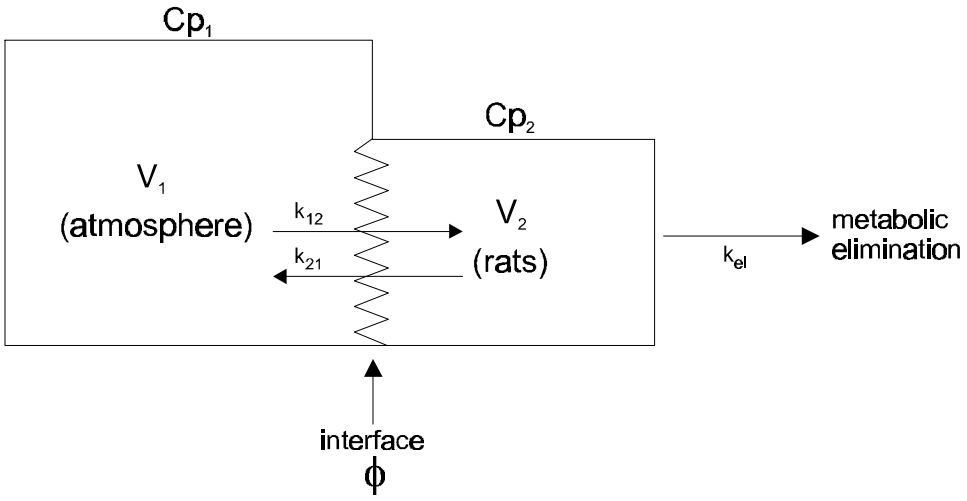

Figure 3-2. Two-compartment pharmacokinetic model for inhalation chamber.

Source: Filser and Bolt, 1981.

Dawley rats and male B6C3F$_1$ mice to 62.5 ppm 1,3-butadiene for 4 h, as described in Table 3-7, with the results shown in Table 3-9. The same group of investigators (Thornton-Manning et al., 1995b) also examined gender differences in the production and disposition of epoxybutene and diepoxybutane by determining tissue concentrations of the two butadiene metabolites in male and female Sprague-Dawley rats, as described in Table 3-7, with the results shown in Table 3-10. The concentrations of epoxybutene did not differ significantly between male and female rats in any of the tissues examined. The highest concentrations were observed in the fat tissues of both sexes. Tissue levels of the diepoxybutane, however, were consistently greater in females than in males. Blood diepoxybutane levels of female rats were 4.75-fold greater than those of male rats. The greatest gender difference was in the levels of the diepoxybutane in fat tissue, with females having a sevenfold greater tissue concentration than males. The mammary tissue of females also contained relatively high levels of the diepoxybutane. The authors suggest that the greater production of the highly mutagenic diepoxybutane in females may play a role in the increased incidence of mammary tumors observed in a chronic carcinogenicity study with rats (Owen et al., 1987).

Dahl et al. (1991) exposed cynomolgus monkeys to nose-only inhalation of 1,3-butadiene and measured the levels of diepoxybutane and 3-butene-1,2-diol. Results for diepoxybutane are included in Table 3-9 for comparison to 1,3-butadiene and epoxybutene levels measured in their previous study (Dahl et al., 1990). Exhaled air and excreta were collected during exposure and for 96 h after exposure and are summarized in Table 3-11.

Two in vivo studies provided data on the urinary excretion of butadine metabolites by humans. In the first study (included in Table 3-7 and described in more detail here), Bechtold et al. (1994) identified and measured two metabolites of 1,3-butadiene, 1,2-dihydroxy-4-(*N*-acetylcysteinyl-*S*-)-butane (M-I) and 1-hydroxy-2-(*N*-acetylcysteinyl-*S*-)-3-butene (M-II) in the urine of workers employed at the Texaco Chemical Co. in Port Neches, Texas, a 1,3-butadiene extraction plant. The study population included (1) exposed employees who worked in two areas (described as low- and high-exposure areas) with time-weighted average concentrations of 3 to 4 ppm 1,3-butadiene over the previous 6 months; (2) an intermediate exposure group spending variable time periods in low- and high-exposure areas; (3) nonexposed employees who worked in areas with historical time-weighted average concentrations of less than 0.1 ppm 1,3-butadiene; and (4) outside controls who had no known exposure to 1,3-butadiene. Urine samples were analyzed from 7, 3, 10, and 9 subjects, respectively, from the above four groups. The assay was based on isotope-dilution GC-MS. After addition of deuterated internal standards, the metabolites were isolated from urine samples by solid-phase extraction and selective precipitation. M-I but not M-II could be readily identified and quantitated in the urine samples

Table 3-11. Excretion of ^{14}C by monkeys exposed to 1,3-[^{14}C]-butadiene[a]

Exposure concentration (ppm)	Exhalants			Uptake	
	CO_2	Other[b]	Urine	Feces	Total metabolites recovered[c]
10.1	1.5 ± 0.2	0.45 ± 0.33	0.9 ± 0.1	0.021 ± 0.005	2.88 ± 0.22
310	0.21 ± 0.04[d]	0.40 ± 0.21	0.8 ± 0.2	0.011 ± 0.003[d]	1.40 ± 0.42[d]
7760	0.08 ± 0.02[d,e]	1.00 ± 0.35	0.58 ± 0.06[d]	0.002 ± 0.001[d,e]	1.65 ± 0.29[d]

[a]Values are mean percentage of total inhaled ± SE measured for 96 h after 2-h exposure.
[b]Includes all material (except CO_2) exhaled during the 2-h exposure and 96-h postexposure.
[c]Mean ± SE of the sums of CO_2, other, urine, and feces values for individual monkeys; does not include residues, if any, in monkeys' bodies.
[d]Significantly different from low-level exposure ($p<0.05$).
[e]Significantly different from mid-level exposure ($p<0.05$).
Source: Dahl et al., 1991.

(limits of sensitivity for this assay, 100 ng/mL). The average values of M-I for exposed, intermediately exposed, nonexposed, and outside control employees were 3,200 ± 1,600, 1,390± 550, 630 ± 190, and 320 ± 70 ng/mL, respectively. Although the levels of exposure for each individual were not known, the urinary levels of M-I for the exposed groups were significantly higher ($p<0.05$) compared with the outside control group. The implications of M-I in the urine from individuals with no known exposure to 1,3-butadiene are not known.

In the second study that provided human data, Ward et al. (1996a) reported increased levels of the urinary metabolite 1,2-dihydroxy-4-(*N*-acetyl-cysteinyl)-butane (a human urinary metabolite also idenfified by Bechtold et al., 1994) and somatic mutations in workers at a styrene-butadiene rubber plant. Exposure was assessed in workers from areas of higher exposures (reactor, recovery, tank farm, laboratory) and lower exposure (blend,coagulation, bailers, shipping, utilities, shops) using badge dosimeters; the concentration of the metabolite was measured in urine; and the frequency of *hprt* mutant lymphocytes was determined by autoradiography. The detection limit (0.25 ppm) was exceeded in 20/40 dosimeter readings in the high-exposure group and in 0/20 readings in the low-exposure group. Sixteen high- and nine low-exposure urine and blood samples were analyzed. Expressed as ng/mg creatinine, metabolite concentrations were 2,363 ± 1,880 and 937 ± 583 ($p<0.05$), respectively, for the

high- and low-exposure groups. The respective mean mutant frequencies were $7.09 \pm 5.2 \times 10^{-6}$ and $2.26 \pm 1.34 \times 10^{-6}$ ($p<0.05$).

Other in vivo studies that further confirm the pathways shown in Figure 3-1 but use different intermediate endpoints than those shown or with noninhalation exposure are described below. Deutschman and Laib (1989) studied the effects of 1,3-butadiene exposure on nonprotein sulfhydryl (NPSH) content of lung, heart, and liver tissue of rats and mice. In these experiments, male $B6C3F_1$ mice and Sprague-Dawley rats were exposed to 1,3-butadiene at concentrations of 10, 50, 100, 250, 500, 1,000, or 2,000 ppm for 7 h. For rats, a reduction (≈70%; significance level not stated) occurred in liver NPSH for animals exposed to 1,000 to 2,000 ppm. A reduction of approximately 20% was observed in lung NPSH of rats, and no appreciable depletion of NPSH was observed for heart tissue of rats. For mice, depletion of hepatic NPSH was observed at exposure concentrations of 100 to 250 ppm and declined to 20% of control for the 2,000 ppm exposure group. Similarly, the NPSH content of mouse lung tissue also declined by 80% to 90% at the two highest exposure levels. For heart NPSH content in mice, minor declines were noted for exposure levels up to 500 ppm, but a rapid decrease was observed between 1,000 and 2,000 ppm that resulted in an ≈75% depletion. Kreiling et al. (1988) suggested that the greater susceptibility of mice to the carcinogenic effects of inhaled 1,3-butadiene might reasonably be explained by the higher rate of formation of the epoxide intermediate and its limited detoxification and subsequent accumulation in mice. The authors applied the concentration-response data to the exposures used in earlier bioassays (HLE, 1981) and noted that, for rats exposed chronically to 1,3-butadiene concentrations of 1,000 or 6,000 ppm, a daily hepatic NPSH depletion of about 25% and 60% and lung NPSH content depletion of 20% and 30% for the low and high exposures, respectively, was calculated. However, these values were based on assumptions that 1,3-butadiene metabolism and NPSH resynthesis remained constant throughout the duration of the chronic exposure. Applying the same methods and assumptions for mice, daily NPSH depletions for the low-exposure (625 ppm) and high-exposure (1,250 ppm) levels, respectively, were estimated for liver (50% and 70%), lung (70% and 90%), and heart (25% and 40%). In studies assessing the effects of 1,3-butadiene exposure on NPSH content of various tissues, Deutschmann and Laib (1989) reported that depletion of cardiac NPSH content in mice after inhalation exposure was an indicator of systemically available epoxide intermediates of 1,3-butadiene that reach the heart by efferent blood flow from the lungs or liver.

The reduction and/or depletion of NPSH content in mice is also indicative of saturation of conjugation of the epoxide metabolites of 1,3-butadiene by glutathione. Glutathione conjugation of epoxybutene and metabolism by glutathione S-transferase was shown by Malvoisin et al. (1981) and Bolt et al. (1983), respectively, and a reduction in hepatic NPSH content in mice

exposed to 1,3-butadiene was shown by Kreiling et al. (1987). A more recent study by Kreiling et al. (1988) suggested that glutathione conjugation may be important in the detoxification of this reactive intermediate. Air-exposed control animals exhibited a moderate time-dependent decrease in hepatic NPSH content, whereas those animals exposed to 1,3-butadiene exhibited a significantly greater reduction in NPSH. After 7 h of exposure, the hepatic NPSH content in mice was reduced to approximately 20% and was reduced further to about 4% after 15 h of exposure. For Sprague-Dawley and Wistar rats, hepatic NPSH content was initially reduced to 80% and 65%, respectively, but remained stable thereafter. Concurrent with the significant depletion of NPSH in the mice were signs of acute toxicity (specifics not noted); no toxicity was observed in any of the rats tested. This experiment clearly demonstrates species variability in the magnitude and time course of hepatic NPSH depletion after inhalation exposure to high concentrations of 1,3-butadiene. Furthermore, the progressive decline in hepatic NPSH content in mice correlates with a reduction in epoxide exhalation and a decline in 1,3-butadiene metabolism. The accumulation of epoxide intermediates (epoxybutene and diepoxybutane) in mice (Bond et al., 1986) is consistent with the observed depletion of hepatic NPSH in this species and the increased metabolism (i.e., production of epoxide intermediates) observed for mice.

Nauhaus et al. (1996) indicated that metabolites were detected in mouse urine that are also seen following exposure to acrolein and acrylic acid, suggesting that these compounds may arise directly from 1,3-butadiene oxidation or indirectly from further metabolism of crotonaldehyde. Rats excreted 1,3-dihydroxypropanone, a metabolite that may be derived from hydrolysis of diepoxybutane. Metabolites derived from diepoxybutane were similar in rats and mice when expressed as a percentage of total metabolites; however, when normalized to body weight, the amount of diepoxybutane-derived metabolites was four times greater in mouse urine than in rat urine. The greater body burden of diepoxybutane in the mouse and the greater ability of rats to detoxify diepoxybutane through hydrolysis may be related to the greater toxicity of 1,3-butadiene in mice. The metabolites derived via reactive aldehyde intermediates in mice also suggest a role of these aldehydes in the toxicity of 1,3-butadiene.

Following i.p. injection of 14.3 or 143 µmol/kg of epoxybutene, two glutathione conjugates, *S*-(2-hydroxy-3-buten-1-yl)glutathione (I) and *S*-(1-hydroxy-3-buten-1-yl)glutathione (II), were detected in the bile of rats (Sharer and Elfarra, 1992). At either dose, the amount of conjugates excreted in 30 min was at least 85% of that excreted in 120 min. When the epoxybutene dose was varied between 14.3 and 286 µmol/kg and the combined amounts of conjugates I and II excreted in 60 min were determined, an apparent linear dose-relationship was obtained. Saturation was not observed at these dose levels. Total conjugates excreted in 60 min averaged 7.6% ± 4.2% of the administered dose with approximately a 3:1 ratio of conjugates I:II.

Although the study showed that epoxybutene GSH conjugates are formed in vivo after administration of epoxybutene, biliary excretion of GSH conjugates account for only a small portion of the administered dose.

N-Acetylcysteine derivatives of the two glutathione conjugates of epoxybutene identified in the bile of rats by Sharer and Elfarra (1992) were detected in the urine of rats and mice administered with epoxybutene intraperitoneally (Elfarra et al., 1995). When rats were injected with epoxybutene at doses ranging from 71.5 to 285 µmol/kg, the urinary excretion of *S*-(2-hydroxy-3-buten-1-yl)-*N*-acetyl-L-cysteine (I) and *S*-(1-hydroxy-3-buten-2-yl)-*N*-acetyl-L-cysteine (II) within 8 h of epoxybutene administration exhibited a linear dose-relationship; the total amount of the two mercapturic acids combined averaged 17% ± 4%. No metabolites were detected in urine samples collected 8 to 24 h after dosing. Mice excreted similar amounts of mercapturic acids (26% ± 13%) at 285 µmol/kg within 24 h of dosing. However, at 143 and 71.5 µmol/kg, excretion accounted for only 7% ± 3% and 9% ± 3% of the dose, respectively. Rats preferentially excreted mercapturic acid II over I (approximate ratio 3:1), whereas mice preferentially excreted mercapturic acid I over II (approximate ratio 1.85:1). The study showed that at low exposure levels, rats excrete higher levels of epoxybutene mercapturic acids than mice.

In summary, the inhalation studies show the uptake of 1,3-butadiene exhibits first-order kinetics at exposure concentrations <1,000 ppm, but at higher concentrations, the process becomes saturated and exhibits zero-order kinetics; mice exhibit saturation kinetics at lower exposure concentrations than do rats. At exposure concentrations up to 1,800 ppm, the uptake of 1,3-butadiene is approximately fourfold greater in mice than in rats. In addition, mice accumulate a greater amount of 1,3-butadiene or its metabolites or both than do rats exposed similarly. Limited data on monkeys indicate that the metabolic uptake rate is less than that for rats or mice. After inhalation of 1,3-butadiene, mice appear to have greater levels of radioactivity (15- to 100-fold greater at all time points after exposure) in all tissues than do rats exposed similarly, but no significant qualitative differences have been observed regarding storage depots or target tissues. However, immediately after a 2 h inhalation exposure, mice exhibited higher levels of 1,3-butadiene metabolites (including the reactive epoxybutene) in the blood than did rats. A comparison of butadiene epoxide levels in target tissues (blood, bone marrow, heart, lung, fat, spleen, and thymus) of rats and mice following inhalation of low levels of 1,3-butadiene showed consistently higher epoxide levels in mouse than in rat tissues. Other in vivo experiments demonstrated gender differences in the production of butadiene metabolites in rats, with tissues from female rats containing higher concentrations of the diepoxybutane than tissues from male rats. The experiment also showed that the levels of epoxybutene were similar in males and females. In vivo experiments have confirmed the role of cytochrome P-450 in the metabolic

activation of 1,3-butadiene observed in in vitro studies. Epoxybutene, a reactive intermediate, may undergo epoxide hydrolase-mediated hydroxylation or conversion via P-450 to another reactive intermediate, diepoxybutane. Conjugation with glutathione represents a detoxification process.

Although 1,3-butadiene may be metabolized by microsomal cytochrome P-450 in both rats and mice, species-related quantitative differences in the fate of inhaled 1,3-butadiene are well documented. The greater susceptibility of mice to the carcinogenic effects of 1,3-butadiene may be related to the higher rate of epoxybutene formation and the limited detoxification and, hence, the greater accumulation of this reactive intermediate in this species. At concentrations >2,000 ppm, the metabolism of 1,3-butadiene follows saturation kinetics in both rats and mice, but the rate of metabolism in mice is greater (about twice); furthermore, the metabolism of epoxybutene is saturable in mice but not in rats. With increasing exposure concentration, the metabolic capacity for epoxybutene becomes rate-limiting in mice but not in rats. Data available from studies with nonhuman primates show that at low-exposure concentrations ($\leq$10 ppm), the steady-state tissue levels of reactive 1,3-butadiene metabolites are lower in monkeys than in rats or mice. The lower uptake rate of inhaled 1,3-butadiene by monkeys suggests that, for comparable exposures, monkeys will receive a lower internal dose of reactive butadiene metabolites. The uptake and retention of 1,3-butadiene appears to be nonlinear in the concentration ranges used in long-term exposure studies, and repeated exposures to 1,3-butadiene do not appear to induce its metabolism.

1,3-Butadiene may be excreted via the respiratory tract, urine, or feces. The rate of 1,3-butadiene excretion by rats and mice was shown to be unaffected by exposure concentration (0.14 to 13,000 µg/L). Half-lifes for urinary excretion of radioactivity were similar for both rats and mice (5.6 and 4.6 h, respectively), but fecal excretion was somewhat greater in rats (22 h) than in mice (8.6 h). A shift to excretion of 1,3-butadiene-derived [^{14}C] via the lungs was noted for rats but not mice at high (13,000 µg/L) exposure concentrations. Approximately 2% of the total inhaled dose was excreted as $^{14}CO_2$ or in the urine of monkeys exposed for 2 h to 1,3-[^{14}C]-butadiene at concentrations ranging from 10 to 8,000 ppm. At the higher concentrations, the proportion of CO_2 decreased, whereas exhaled metabolites (diepoxybutane and butene diol) increased. Elimination of radioactivity from the blood and tissues of rats and mice after inhalation exposure to 1,3-[^{14}C]-butadiene was biphasic; half-lifes for initial removal were 2 to 10 h and for slower elimination were 5 to 60 days. Excretion of epoxybutene via the lungs by rats and mice also has been studied and notable differences between the species observed. For rats, exhaled epoxybutene concentrations at 10 h attained a plateau of about 4 ppm and remained at this level for >12 h. For mice, however, the plateau level was about 10 ppm but declined to 6 ppm at 15 h, a decline that coincided with signs of acute toxicity in the mice.

Studies on the urinary excretion of 1,3-butadiene metabolites in mice, rats, hamsters, monkeys, and humans have shown that all these species predominantly produce two urinary metabolites, 1,2-dihydroxy-4-(*N*-acetylcysteinyl-*S*)-butane (M-I) and 1-hydroxy-2-(*N*-acetylcysteinyl-*S*)-3-butene (M-II), but in different proportions. The M-II is a mercapturic acid formed by conjugation of GSH with epoxybutene, while M-I is a mercapturic acid that appears to form by GSH conjugation with butene diol, the hydrolysis product of diepoxybutane. M-I but not M-II was also found in the urine of workers exposed to low levels of 1,3-butadiene.

3.2. MOLECULAR DOSIMETRY

In addition to data on absorption, metabolism, and excretion, a complete dosimetry model for 1,3-butadiene should incorporate information on molecular dosimetry, which links exposure to some internal biomarkers of exposure. This last component is best evaluated by assessing adduct formation.

The use of Hb adducts as biomarkers of exposure to 1,3-butadiene was investigated by Sun et al. (1989a). In this study, male B6C3F$_1$ mice and male Sprague-Dawley rats were injected intraperitoneally with 1,3-[^{14}C]-butadiene at doses of 1, 10, 100, or 1,000 µmol/kg, and adduct formation was monitored. Hb adduct formation was linearly related to dose up to 100 µmol/kg for both species. The Hb adducts accumulated linearly after repeated injections of 100 µmol/kg for 3 days. The 1,3-butadiene-derived Hb adducts showed lifetimes of ≈24 and ≈65 days in mice and rats, respectively, which correlates with the lifetimes of red blood cells. Assuming that adduct formation is a function of the extent of 1,3-butadiene metabolism, the similarity in the degree of Hb adduct formation between mice and rats does not reflect the species variability in toxicity of this compound. Therefore, Hb adducts may not serve as accurate indicators of levels of reactive metabolites in the blood and, thus, as indicators of toxicity. However, Hb adduct formation may be useful as an indicator of 1,3-butadiene exposure.

Similar findings of exposure-dependent Hb adduct formation and stability of the adducts were reported by Osterman-Golkar et al. (1991) for Wistar rats exposed to 1,3-butadiene at concentrations of 250, 500, or 1,000 ppm, 6 h/day, 5 days/week for 2 weeks. In this study, the Hb adduct formation also increased linearly with exposure up to the highest exposure level. The investigators also concluded that Hb adducts were useful for assessing dosimetry of long-term exposure to 1,3-butadiene.

Osterman-Golkar et al. (1996) studied Hb adducts in 17 workers exposed to 1,3-butadiene in a petrochemical plant and nine referents employed at the same factory but not exposed to 1,3-butadiene. Using stationary and personal monitoring devices, the ambient 1,3-butadiene level for workers handling 1,3-butadiene containers was 11.2 ± 18.6 mg/m^3 and ≤1.2 mg/m^3 for maintenance and laboratory workers. The Hb adduct measured was 2-hydroxy-3-butylvaline,

formed by reaction of *N*-terminal valine with carbon 1 in epoxybutene. Higher concentrations of Hb adducts (0.16 ± 0.099 pmol/g) were recorded in the workers handling 1,3-butadiene containers compared with those in maintenance, laboratory workers, and nine unexposed controls (≈0.05 pmol/g).

Citti et al. (1984) conducted an in vitro study that examined the reactivity of epoxybutene (referred to as epoxybutene by the authors) with isolated nucleosides and DNA. They reported that two adducts were formed: 7-(2-hydroxy-3-buten-1-yl)guanine and 7-(1-hydroxy-3-buten-2-yl)guanine. The authors indicated that the epoxide reacted similarly with either free DNA or DNA-bonded deoxyguanosine and that the half-life of these adducts under physiological conditions was 50 h.

Kreiling (1987) reported the in vivo formation of the DNA adduct 7-(1-hydroxy-3-buten-2-yl)guanine in the liver of mice exposed to 1,3-[^{14}C]-butadiene (exposure concentration and duration not specified). No DNA adducts were detected in the livers of 1,3-butadiene-exposed rats. Note that this adduct was one of two reported by Citti et al. (1984) for the in vitro reaction of 3,4-epoxybutene with DNA and deoxyguanosine. Additional details were not available in the abstract by Kreiling nor was additional information reported in later publications by Kreiling and coworkers.

Jelitto et al. (1989) reported species-dependent differences in the in vivo formation of DNA adducts by male B6C3F$_1$ mice and male Sprague-Dawley rats exposed to 1,3-[^{14}C]-butadiene at concentrations of 250, 500, or 1,000 ppm for 7 h. Analysis (alkaline elution and comparison of HPLC profiles with synthesized adduct standards) of liver DNA from the mice showed that two adducts had been formed: 7-*N*-(1-hydroxy-3-buten-yl)guanine and 7-*N*-(2,3,4-trihydroxybutyl)guanine, the latter being derived from diepoxybutane. These products were not detected in rat liver DNA. Alkaline elution curves showed that protein-DNA and DNA-DNA cross-linking occurred in mice, but not in rats, after a 7 h exposure to 1,3-butadiene at concentrations of 250 ppm and above. These findings provide additional evidence at the molecular level for explaining the difference in the carcinogenic response between mice and rats.

3.3. STRUCTURE-ACTIVITY RELATIONSHIPS

Studies by Del Monte (1985) and Dahl et al. (1987) have shown that the metabolism of structurally related isoprene (2-methyl-butadiene) may be qualitatively similar to that of 1,3-butadiene. Although the diepoxybutane metabolite of isoprene has been shown to be genotoxic in *Salmonella*, data are unavailable regarding the carcinogenic potential of isoprene.

Del Monte et al. (1985) showed that mouse hepatic microsomal monooxygenases converted isoprene to epoxides and diepoxides and that the biotransformation was inhibited by cytochrome P-450 inhibitors such as CO, SKF 525-A, and metyrapone. Specifically, 3,4-epoxy-3-methyl-

butene and 3,4-epoxy-2-methyl-1-butene were major and minor metabolites, respectively, with the latter representing about 20% of the former. The 3,4-epoxy-2-methyl-1-butene metabolite was metabolized further in microsomal incubations to the mutagenic isoprene dioxide (diepoxide). Data from these in vitro metabolism studies were used to calculate the K_M and V_{max} for the production of the diepoxide. The resulting K_M (mM) and V_{max} (nmol diepoxide/mg protein/min) values for diol production by microsomes from control, phenobarbital-induced, and 3-methylcholanthrene-induced mice were 0.24 and 1.7, 0.29 and 5.1, and 0.22 and 2.0, respectively. The V_{max} for the formation of the diepoxide was significantly increased ($p<0.01$) in incubations using hepatic microsomes from phenobarbital-treated mice.

Gervasi and Longo (1990) provided additional information on the metabolism of in vitro isoprene by hepatic microsomal preparations from rats, mice, rabbits, and hamsters. Hepatic microsomal preparations from these species metabolized isoprene to epoxybutene, 3,4-epoxy-3-methyl-1-butene, and 3,4-epoxy-2-methyl-1-butene. The former was the major metabolite and was found to have a half-life of 85 min. Microsomal preparations from all species further metabolized the 3,4-epoxy-2-methyl-1-butene to isoprene dioxide (2-methyl-1,2,3,4-diepoxybutane), which was found to be mutagenic and to have alkylating ability. The K_M (mM) and V_{max} (nmol/mg/protein/h) for the rat, mouse, rabbit, and hamster microsomal metabolism of isoprene were 0.08 and 0.24, 0.09 and 1.79, 0.2 and 0.66, and 0.06 and 1.20, respectively. Unlike 1,3-butadiene, isoprene exhibited the same pattern of metabolism in all species tested and did not result in mutagenic epoxybutene intermediates.

In the study by Dahl et al. (1987), groups of 30 male F344 rats were exposed by nose-only inhalation to [^{14}C]isoprene at concentrations of 8.0, 266, 1,480, or 8,200 ppm for 6 h (5.5 h for the highest exposure), and urine, feces, and exhalants were collected over a 66 h postexposure period. During this period, >75% of the nonisoprene (metabolites) radioactivity was excreted in the urine. Except for the highest exposure group where greater amounts of radioactivity were excreted in the feces, a pattern of predominantly urinary excretion was consistent among the various exposure groups. The half-life (mean ± SE) for urinary excretion of ^{14}C was 10.2 ± 1.0 h (range of 8.8 to 11.1 h). Generally, the concentration of metabolites in the blood increased with exposure concentration and duration of exposure. The authors noted that 85% of the radioactivity in the blood was associated with material of low volatility and that it probably represented covalently bound metabolites, conjugates of isoprene metabolites, or tetrols. Only at the two highest exposure concentrations were materials detected that possessed volatilities matching those of isoprene and isoprene monoepoxides. The percentage of inhaled isoprene-derived ^{14}C present as diepoxide or diol in the blood remained fairly constant with time but decreased with exposure concentration. Assessing the distribution of isoprene and its metabolites in some animals of the 1,480 ppm exposure group revealed that the liver and blood

contained the majority of the radioactivity. Relatively large amounts of metabolites were present in respiratory tract tissues after 20 min of exposure. The mutagenic metabolite, isoprene diepoxide, was identified in all tissues examined and, in the blood, represented between 0.0018% and 0.031% of the inhaled ^{14}C label. Although exposure to high concentrations of 1,3-butadiene result in CO_2 as the major metabolite, this study suggested that the major route of excretion for isoprene is in the urine. The authors noted, however, that this finding is tentative and may be the result of a labeling artifact. Although no evidence for metabolic saturation was detected for the isoprene concentrations used, the uptake and fate of inhaled isoprene are similar to that of butadiene.

Peter et al. (1987) also studied the pharmacokinetics of isoprene in male Wistar rats and male $B6C3F_1$ mice. Animals were exposed in closed systems to concentrations as high as 4,000 ppm for up to 10 h. At concentrations <300 ppm, the rate of metabolism was found to be directly proportional to the isoprene concentration, but saturation of metabolism was detected at higher concentrations. The V_{max} for the metabolism of isoprene in rats and mice was 130 and 400 µmol/h/kg, respectively. Exhalation of the parent compound was approximately 15% and 25% in rats and mice, respectively.

Chloroprene (2-chloro-butadiene) is also structurally similar to 1,3-butadiene. Studies have shown that the biotransformation of chloroprene results in the formation of peroxides that may interact with tissue thiols (Haley, 1978). Furthermore, cytochrome P-450 mixed-function oxygenases may form an epoxide intermediate similar to that formed during 1,3-butadiene metabolism.

In summary, in vitro metabolism studies have shown that the structurally similar isoprene is metabolized in a similar fashion by several different species and that epoxybutene intermediates are formed, one of which may be epoxidized further to a genotoxic diepoxybutane. In vivo inhalation studies that used rats and mice exposed to isoprene showed that its uptake and fate are similar to that of 1,3-butadiene and that a genotoxic diepoxybutane metabolite, but not a genotoxic epoxybutene intermediate, is formed.

Preliminary data indicate that Hb adducts may be useful as biomarkers of exposure for 1,3-butadiene exposure. Research efforts are focusing on dosimetry modeling for extrapolating from high- to low-dose exposures and for interspecies extrapolation. Furthermore, on validation, dosimetry models may be useful in predicting levels of 1,3-butadiene and its reactive metabolites in various tissues.

3.4. DISCUSSION AND CONCLUSIONS

Species variability in the metabolism and disposition of 1,3-butadiene may explain, in part, species variability in the toxicity of the compound. Current data indicate that the toxicity of 1,3-

butadiene depends on the metabolic activation to reactive intermediates such as epoxybutene and diepoxybutane and that these biotransformation processes vary quantitatively and qualitatively among species. The mutagenic epoxybutene and diepoxybutane metabolites have been shown to occur in the blood of rats and mice exposed to 1,3-butadiene, and their concentrations are two- to fivefold greater in the blood of mice. Limited data for humans have shown that liver microsomes have a higher capacity for the formation of epoxybutene than do rodent liver microsomes but that the metabolism of epoxybutene to 1,3-butadiene epoxide by human liver microsomes was 20-fold greater than that observed in rat or mouse microsomes. These data suggest that levels of this reactive intermediate in humans may be substantially less than in the rodent species. The oxidation of epoxybutene to diepoxybutane (also a reactive metabolite) appears to be negligible in humans and rats (formation of the non-DNA-reactive butene diol 1,2-dihydroxybut-3-ene is the preferred pathway) and is substantial in mice. Study results have shown species-related differences in the uptake and retention of inhaled 1,3-butadiene. Uptake and retention by mice is greater than for rats, and saturation kinetics are observed in mice at exposure concentrations of 500 ppm but not in rats at exposures as high as 5,000 ppm. These differences may be used to support the hypothesis that the greater sensitivity of mice to the toxic effects of 1,3-butadiene may be a function of a greater internal dose, greater production of reactive metabolites, and lower detoxification potential.

Although the previous findings provide considerable insight into the understanding of 1,3-butadiene toxicity, some researchers have indicated the need for examining additional, although quantitatively minor, metabolic pathways (e.g., glutathione S-transferase-mediated detoxification processes and formation of toxic metabolites such as butene diol and crotonaldehyde) and the possible effects of pulse exposures on the metabolism and disposition of 1,3-butadiene.

Molecular dosimetry studies have also shown species-related differences in the formation of various adducts. Additional work in this area will be useful in assessing these adducts as either biomarkers of exposure or effects.

Dosimetry models are being developed or refined to extrapolate the relatively high exposures and doses used in animal tests to the low exposure concentrations in human exposure situations. These models will be especially useful in predicting blood and tissue concentrations of butadiene metabolites.